



Original Article

Autonomic-cyclooxygenase-2 Mechanisms of Electroacupuncture on Thermal Injury-induced Gastric Dysmotility in Rats



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Abstract

Background and objectives: Gastrointestinal dysmotility commonly follows thermal injuries, such as burns. This study aimed to investigate the effects and mechanisms of electroacupuncture (EA) on burn-induced gastric dysmotility in rats.

Methods: Sprague-Dawley rats were divided into sham and thermal injury groups subjected to a 60% scald burn. Antagonists, including β -blockade (propranolol), α -blockade (phentolamine), or a selective cyclooxygenase (COX)-2 inhibitor (nimsulide), were administered to verify the pathways involved. Six hours after the burn, the animals were evaluated for gastric emptying and heart rate variability. Blood and gastric tissues were collected for assays of cytokines, hormones, and COX-2 levels. EA was performed at bilateral ST36 (Zusanli) acupoints for 45 m.

Results: Burn injury delayed gastric emptying by 61% ($P < 0.01$), which was normalized by nimsulide or propranolol but not by phentolamine. EA improved gastric emptying by 87% ($P = 0.03$) in burned rats. Heart rate variability and plasma hormone (noradrenaline and pancreatic polypeptide) analyses indicated sympathetic hyperactivity in burned rats; EA improved burn-induced sympathovagal imbalance by enhancing vagal activity. Protein and mRNA expressions of COX-2 in the gastric fundus and antrum increased with burn but were normalized by propranolol. EA reduced the burn-induced increase in COX-2 expression in the gastric fundus but not in the antrum. EA also decreased burn-induced elevations in plasma interleukin (IL)-6 and IL-10. Negative correlations were found between gastric emptying and plasma IL-6 levels, as well as between gastric emptying and COX-2 mRNA levels.

Conclusions: These findings suggest that burn-induced gastric dysmotility is mediated via autonomic-COX-2 pathways. EA at acupoint ST36 improves burn-induced delays in gastric emptying by down-regulating COX-2 and pro-inflammatory cytokines through the autonomic nervous pathway.

Introduction

Gastrointestinal dysmotility is a common complication in patients following thermal injuries, such as cutaneous burn injury. Our previ-

ous studies verified the presence of burn-induced delays in gastric emptying in rodents.^{1,2} The clinical benefits of early oral and enteral feeding after burns have been well established; these include increased intestinal blood flow and better maintenance of the gut mucosal barrier, which reduces the risk of bacterial and endotoxin transfer from the gut and subsequent sepsis.^{3,4} However, post-burn delays in gastric emptying remain a frequent obstacle, preventing early enteral resuscitation. Consequently, treating burn-induced gastrointestinal dysmotility is strongly recommended. Given the limitations of current prokinetic medical therapies, exploring novel treatments for impaired gastric motility following burns is essential.

Burn injury alters gastrointestinal motility through the activation of a post-burn inflammatory cascade^{5,6} involving elevated levels of pro-inflammatory mediators such as interleukin (IL)-1 β ,

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IL-6, and tumor necrosis factor- α . Another significant effect of burn injury is the increased production of prostaglandins, which alter both the amplitude and frequency of gut contractions.^{7,8} Prostaglandins are synthesized by two cyclooxygenase (COX) isoenzymes: COX-1 and COX-2. The constitutive isozyme COX-1 mediates physiological responses and is expressed in most cell types, whereas the inducible form COX-2 is undetectable under normal conditions and is rapidly induced in response to stimuli such as inflammation and stress.^{9,10} Numerous studies indicate that COX-2 contributes to gastrointestinal dysmotility under various pathological conditions, including post-operative ileus¹¹ and inflammation.⁹ Elevated COX-2 and immune dysfunction have been reported to play a critical role in gastrointestinal dysmotility induced by burn injury, and our previous studies demonstrated that COX-2 inhibitors could restore burn-induced gut dysmotility.^{1,7,12,13} Taken together, these investigations highlight the role of the COX-2 pathway in burn-induced gastrointestinal dysmotility.

Activation of the sympathetic nervous system is also a well-known feature of burn injury¹⁴ and may subsequently activate a stress-related cascade. The correlation of COX-2 with sympathetic activity has been widely studied; activation of the peripheral sympathetic nervous system and noradrenaline increases COX-2 expression and prostaglandin production.^{15,16} Moreover, sympathetic overactivity contributes to immune dysfunction under stress, as adrenergic receptor blockade reduces elevated cytokines.^{17,18} Additionally, the regulatory effect of the sympathetic nervous system on gastrointestinal motility has been documented; norepinephrine inhibits gastric emptying and small intestinal motility.^{19,20} These findings suggest the involvement of the sympathetic nervous system in burn-induced gastric dysmotility, either directly or via up-regulation of inflammatory cytokines and prostaglandins.

Electroacupuncture (EA), a combination of electrical stimulation and acupuncture, is a traditional Chinese medicine therapy performed by inserting a thin stainless-steel needle into specific acupoints. EA at various acupoints has different therapeutic effects and is used for various medical purposes, including pain, obesity, and gastrointestinal disorders.^{21–26} ST36 (Zusanli) is the most commonly used acupoint for treating gastrointestinal diseases. Previous studies have reported that EA at ST36 improves gastrointestinal dysmotility under various conditions, such as postoperative ileus, functional dyspepsia, and gastroparesis.^{27–29}

Recent studies revealed the involvement of autonomic nervous pathways in the effects of EA on gut motility. The prokinetic effect of EA on gastric emptying was found to be mediated via the vagal pathway. EA increased vagal activity,^{29,30} activated vagal afferent neurons in the brainstem, and became ineffective when vagotomy was performed.^{31,32} Nevertheless, the detailed signaling pathways involving autonomic function in the acceleratory effect of EA on gastric motility still need to be delineated.

Although EA has been reported to improve gastrointestinal motility, its effect on burn-induced dysmotility and the underlying molecular mechanisms have not yet been investigated. The aim of this study was to investigate the ameliorating effect and autonomic-COX-2 mechanisms of EA on burn-induced gastric dysmotility in rats.

Materials and methods

Animal preparation and experimental design

Adult male Sprague-Dawley rats (300–350 g) were acclimated under conditions of 22°C with a 12-h light/dark cycle for one week prior to the experiments. A total of 69 rats was included in this

study and randomly divided into eight groups: one sham burn group (n = 10) and seven burn groups: untreated (n = 10); EA-treated (n = 10); sham-EA1 (n = 10, one rat was excluded due to technical issues); sham-EA2 (n = 6); propranolol-treated (n = 8); phenolamine-treated (n = 8); and nimsulide-treated (n = 8). The smaller number of rats in the sham-EA2 group was determined by preliminary testing, which showed ineffectiveness of sham-EA. The sample size was estimated based on burn-induced gastric emptying data from a previous study. The animal study was performed in compliance with the guidelines of the National Institutes of

Health and approved by the Animal Care and Use Committee of the University of Texas Medical Branch.

Under general anesthesia with 2% isoflurane (Abbott Laboratories, North Chicago, IL, USA), animals underwent surgical implantation of three subcutaneous electrodes for electrocardiogram (ECG) measurement: two electrodes were affixed to the chest muscles, and a third was affixed to the abdominal muscles. Animals were allowed to recover for three days prior to burn or sham injury.

Burn injury was inflicted according to the Walker-Mason burn model, inducing a full-thickness (3rd degree) scald burn.³³ Following a 24-h fast, a 60% total body surface area scald burn or sham burn was inflicted under deep general anesthesia with 5% isoflurane as previously described.^{1,2} In brief, the dorsum and ventral surface of the abdomen were shaved, and the rats were placed in a mold with the defined areas exposed and immersed in 95°C water, scalding the dorsum for 10 s and the ventral surface for 2 s. Both front and hind legs were protected to avoid any burn injury. Immediate resuscitation after burn injury was performed by intraperitoneal injection of Ringer lactate solution according to the Parkland formula (4 mL/kg/% total body surface area/24 h). Sham-burn rats were treated identically except the water temperature was 25°C. After the burn, rats had no access to food or water.

Six hours after the sham or burn injury, rats were offered a solid meal and subjected to EA or sham EA for 45 m. Heart rate variability (HRV) was recorded for the first 30 m in the postprandial state. Ninety minutes after the meal, animals were sacrificed; blood and stomachs were collected.

Drug administration

Antagonists were administered intraperitoneally to verify the pathway. All antagonists were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in 0.5 mL of 10% DMSO. An equal amount of vehicle was injected in the corresponding control session. Phenolamine (alpha-adrenoceptor antagonist; 5 mg/kg) or nimsulide (selective COX-2 inhibitor; 10 mg/kg) was injected immediately after burn injury. Propranolol (beta-adrenoceptor antagonist; 10 mg/kg) was injected twice, once at the time of the burn and again six hours following burn injury.^{2,34,35}

Gastric emptying

Six hours after the sham or burn injury, rats were fed with 1.5–2.0 g of regular chow pellets within 5–10 m. Ninety minutes later, the rats were euthanized by decapitation under deep general anesthesia with 5% isoflurane. The stomach was collected, and the residual food in the stomach was scraped out, dried, and weighed. Gastric emptying was calculated as previously described: Gastric emptying (%) = (1 - dried weight of food recovered from stomach/weight of food intake) $\times$ 100.³⁶

EA treatment

Following meal consumption, EA was applied at bilateral stomach-36 (ST-36), located 5 mm below the head of the fibula and 2

mm lateral to the anterior tubercle of the tibia, for 45 m. Needles were inserted into ST36 to a depth of 3–5 mm and connected to an electrical stimulator (PulseMaster A300, WPI, Sarasota, USA). Stimulation parameters shown to have prokinetic effects on gastric motility in previous studies were used (pulse train: 2 s on, 3 s off, 25 Hz pulse frequency, 0.5 ms pulse width, and 3–5 mA intensity).^{25,29,30} Two sham-EA groups were tested: in the sham-EA1 group, needles were inserted into non-acupoints and connected to the stimulator with power off; in the sham-EA2 group, the stimulator was turned on. Preliminary data indicated that both sham-EA1 and sham-EA2 had no effects on gastric emptying in burned rats. Subsequently, only sham-EA1 was used.

Assessment of autonomic nervous system

Measurement of HRV

The ECG electrode wires were connected to an amplifier (model 2283 Fti Universal Fetrode Amplifier, UFI, Morro Bay, CA). The ECG was recorded for 30 m in the postprandial state. HRV data were derived from the ECG recording using validated software in our lab.²⁹ Briefly, R waves were identified, and R-R intervals were calculated. R-R interval data were interpolated at 100 Hz and down-sampled to 8 Hz for analysis. The spectral analysis of HRV data was performed using a well-established method: spectral power in the low-frequency band (LF; 0.3–0.8 Hz) represents mainly sympathetic activity, while power in the high-frequency band (HF; 0.8–4.0 Hz) represents purely parasympathetic or vagal activity.²⁹ The LF/HF ratio was calculated to reflect the sympatho-vagal balance.

Measurements of norepinephrine (NE) and pancreatic polypeptide (PP)

Both hormones were measured as plasma NE level correlates with sympathetic activity,²⁹ while plasma PP level reflects vagal activity.³⁶ Blood was collected immediately after decapitation in EDTA-coated tubes. Plasma was separated by centrifugation for 20 m at 1000×g at 4°C within 30 m of collection and stored at –80°C until further analysis. Enzyme immunoassay was used for quantitative determination of plasma NE (LDN, Nordhorn, Germany) and PP (Novatein Biosciences, Cambridge, MA, USA).

Measurement of cytokines

Quantitative sandwich enzyme immunoassay (R&D Systems Inc, Minneapolis, MN, USA) was used to analyze plasma levels of the pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10.

COX-2 expression

The gastric fundus and antrum were immediately frozen in liquid nitrogen and stored at –80°C until further analysis. The protein and mRNA expression of COX-2 were measured by standard methods as previously described.³⁷

Western blot

Gastric tissues were powdered in a mortar with liquid nitrogen, homogenized on ice in lysis buffer [20 mM Tris-HCL (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na₃VO₄, 1 μg/mL leupeptin, and 1/100 proteinase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA)]. Proteins were separated on NuPAGE 4–12% Bis-Tris Gels (Life Technologies, Carlsbad, CA, USA), transferred to Nitrocellulose membranes

(BioRad, Hercules, CA), and blocked in blocking buffer (LI-COR Biosciences) for 1 h at room temperature. Membranes were incubated with anti-COX-2 antibody (1:500, Cayman Chemical, Ann Arbor, MI, USA) at 4°C overnight, then washed 5 times for 5 m with Tris-buffered saline with 0.1% Tween® 20 detergent (TBST), and incubated with Alexa Fluor 680 goat anti-rabbit IgG (1:5,000, Invitrogen) at room temperature for 1 h. β-actin served as a loading control with primary antibody at 1:5,000 (Sigma-Aldrich, St. Louis, MO, USA) and secondary antibody IRDye 800-conjugated anti-mouse IgG (Rockland, Gilbertsville, PA). After washing, membranes were scanned and bands analyzed using Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

Real-time quantitative reverse-transcription polymerase chain reaction

The Qiagen RNeasy kit (Qiagen, Valencia, CA) was used to extract total RNA from frozen tissues. Equal amounts of total RNA (1 μg) were reverse transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen). Real-time PCR was performed using a StepOne Plus thermal cycler (Applied Biosystems, Foster City, CA). TaqMan primers and probes specific for the rat COX-2 gene (Applied Biosystems, Foster City, CA, USA) were used in this study to amplify COX-2, with 18S rRNA also quantified as an endogenous reference. Seven μl (2 μl for 18 s) of each sample's diluted cDNA (1:5) was assayed in triplicate in an Optical 96-well reaction plate. Relative amounts of COX-2 mRNA were calculated using the comparative Ct (ΔΔ CT) method with 18 s rRNA as a normalizer.

Statistics

All data are presented as mean ± SE. Differences were compared using ANOVA followed by post hoc analysis for multiple groups and Student's *t*-test for two groups. Pearson's correlation analysis was used to measure the relationship between gastric emptying and cytokine levels or COX-2 expression. A *P*-value < 0.05 was considered statistically significant.

Results

Effects of burn and EA on gastric emptying

Burn decreased gastric emptying by more than 61% ($21.3 \pm 3.6\%$ vs. $54.4 \pm 9.1\%$ for sham-burn, $P = 0.01$). Nimsulide normalized the delayed gastric emptying ($53.5 \pm 7.5\%$, $P < 0.01$ vs. burn; $P = 0.94$ vs. sham-burn), while propranolol greatly improved it ($44.5 \pm 2.8\%$, $P < 0.01$ vs. burn; $P = 0.36$ vs. sham-burn). Phentolamine had no effect on the delayed gastric emptying ($15.2 \pm 2.7\%$, $P = 0.20$ vs. burn) (Fig. 1a). These findings indicate the involvement of COX-2 and the autonomic nervous system, primarily the β-adrenergic pathway, in burn-induced delay in gastric emptying.

EA accelerated the delayed gastric emptying by 87% ($39.9 \pm 6.7\%$, $P = 0.03$ vs. burn). Neither Sham-EA1 ($18.0 \pm 3.1\%$, $P = 0.50$ vs. burn) nor Sham-EA2 ($17.4 \pm 1.9\%$, $P = 0.41$ vs. burn) had any effect on the burn-induced delay in gastric emptying (Fig. 1b). There was no significant difference in gastric emptying between the two Sham-EA groups.

COX-2 mechanisms in burn-induced delayed gastric emptying and the prokinetic effect of EA

Burn substantially increased the protein and mRNA expressions of COX-2 in both the gastric fundus and gastric antrum ($P < 0.05$ each vs. sham-burn) (Figs. 2a and 3a). In the fundus, the relative

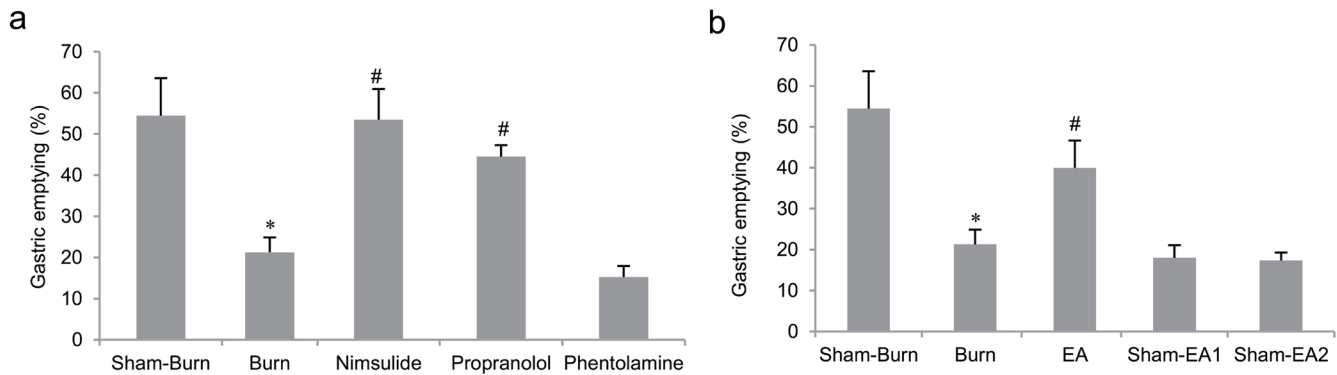


Fig. 1. Effects of EA on gastric emptying in burned rats. (a) The percentage of gastric emptying decreased with burn injury (* $P = 0.01$ vs. Sham-burn) and was significantly improved with nimsulide (* $P = 0.001$ vs. burn) or propranolol (* $P < 0.01$ vs. burn), but not with phentolamine. (b) Gastric emptying significantly increased with EA treatment (# $P = 0.03$ vs. burn), but not with Sham-EAs. EA, electroacupuncture.

amount of COX-2 mRNA was 4.72 ± 0.89 in burn vs. 1.79 ± 0.4 in sham burn ($P = 0.01$), while in the antrum, it was 3.29 ± 0.7 in burn vs. 1.55 ± 0.22 in sham burn ($P = 0.04$). Propranolol normalized COX-2 protein and mRNA expression in both fundus and antrum ($P < 0.05$ vs. burn each; $P > 0.05$ vs. sham-burn each). The relative COX-2 mRNA amounts were reduced to 1.86 ± 0.18 in the fundus ($P = 0.01$ vs. burn; $P = 0.90$ vs. sham-burn) and 1.4 ± 0.18 in the antrum ($P = 0.03$ vs. burn; $P = 0.61$ vs. sham-burn), respectively.

EA significantly decreased burn-induced increases in protein and mRNA expressions of COX-2 in the gastric fundus ($P = 0.01$ and 0.02 , respectively, vs. burn; Fig. 2b). The relative amount of COX-2 mRNA was reduced by 50%, from 4.72 ± 0.89 to 2.12 ± 0.37 , comparable to sham-burn ($P = 0.02$ vs. burn; $P = 0.55$ vs. sham-burn; Fig. 3b). This inhibitory effect was not observed with sham-EA1. However, in the gastric antrum, EA had no significant effect on the burn-induced increase in protein and mRNA expression of COX-2 ($P = 0.14$ and 0.52 , respectively; Fig. 2c).

Gastric emptying was negatively correlated with COX-2 mRNA levels in the gastric fundus ($r = -0.48$, $P < 0.01$), but not with those in the gastric antrum ($r = 0.22$, $P = 0.14$).

Involvement of IL-6 in burn-induced gastric dysmotility and prokinetic effect of EA

There was a dramatic 385% increase in plasma IL-6 levels at 6 h post-burn injury (515.5 ± 69.3 pg/mL vs. 106.2 ± 16.2 pg/mL for sham-burn, $P < 0.01$). This increase was substantially suppressed by propranolol (230.58 ± 16.33 pg/mL, $P < 0.01$ vs. burn; $P = 0.22$ vs. sham-burn) and nimsulide (239.7 ± 13.9 pg/mL, $P < 0.01$ vs. burn; $P = 0.17$ vs. sham-burn; Fig. 4a).

Concurrent with the IL-6 increase, IL-10 also rose more than 10-fold at 6 h after burn injury (51.47 ± 12.35 pg/mL vs. 5.36 ± 0.91 pg/mL for sham-burn, $P < 0.01$). However, this burn-induced increase in IL-10 was not suppressed by propranolol or nimsulide ($P = 0.48$ and 0.98 , respectively, vs. burn; $P = 0.10$ and 0.03 , respectively, vs. sham-burn; Fig. 4b).

EA reduced burn-induced increases in plasma IL-6 by 36%, down to 328.7 ± 52.2 pg/mL ($P < 0.05$ vs. burn), and reduced plasma IL-10 by 64%, to 18.54 ± 5.12 pg/mL ($P = 0.03$ vs. burn). Sham-EA1 did not show an inhibitory effect on burn-induced increases in plasma IL-6 or IL-10 ($P > 0.05$ each vs. burn; Fig. 4c and d).

There was a negative correlation between gastric emptying and plasma IL-6 levels ($r = -0.57$, $P < 0.01$), but not with plasma IL-10 levels ($r = -0.19$, $P = 0.16$).

Autonomic pathways involved in burn-induced gastric dysmotility and the prokinetic effect of EA

Sympathetic hyperactivity was observed in rats following severe burn injury. A significant increase in plasma NE was found in rats 6 h post-burn injury compared to sham-burn rats (72.00 ± 1.78 vs. 56.35 ± 4.50 ng/mL, $P = 0.01$). Propranolol and nimsulide restored plasma NE levels in burned rats to the sham-burn level, reaching 59.45 ± 4.38 ng/mL ($P = 0.01$ vs. burn; $P = 0.63$ vs. sham-burn) and 55.25 ± 2.63 ng/mL ($P < 0.001$ vs. burn; $P = 0.88$ vs. sham-burn), respectively (Fig. 5a).

Sympathetic dominance was also observed in the spectral analysis of HRV derived from the ECG signal. Both the LF component in the HRV power spectrum, representing mainly sympathetic activity, and the LF/HF ratio, representing sympathovagal balance, were greatly elevated in burned rats compared to sham-burned rats (LF: 13.17 ± 4.14 vs. 0.37 ± 0.16 , $P < 0.01$; LF/HF: 9.26 ± 1.53 vs. 2.18 ± 0.69 , $P < 0.01$; Fig. 6). Propranolol significantly decreased LF and improved LF/HF in burned rats (LF: 2.12 ± 0.33 , $P = 0.02$ vs. burn; LF/HF: 5.42 ± 1.06 , $P = 0.02$ vs. burn).

Burn injury did not alter vagal activity. As there was no difference in plasma PP levels between the burn and sham-burn groups (199.01 ± 10.56 pg/mL vs. 197.46 ± 14.37 pg/mL). Propranolol and nimsulide also did not affect plasma PP levels (Fig. 5b).

EA significantly enhanced the HF component in the HRV spectrum, representing vagal activity (2.79 ± 0.54 vs. 1.22 ± 0.32 for burn, $P = 0.03$), and significantly decreased the LF/HF ratio (3.77 ± 0.41 , $P < 0.01$ vs. burn) in burned rats (Fig. 5b). EA also evoked a 44% elevation in plasma PP in burned rats (287.33 ± 30.26 pg/mL, $P = 0.02$ vs. burn), while EA did not affect plasma NE levels.

Discussion

To our knowledge, this is the first study to investigate the molecular mechanisms of COX-2, IL-6, and the autonomic pathways involved in thermal injury-induced gastric dysmotility, as well as the prokinetic effect and mechanisms of EA in treating thermal injury-induced gastric dysmotility in rats. We found that: 1) burn injury delayed gastric emptying, with the burn-induced delay mediated by COX-2 via the sympathetic pathway, and 2) EA at ST36 improved burn-induced delayed gastric emptying via the COX-2 mechanism and the autonomic pathway.

This study demonstrated that severe cutaneous burn injury decreased the gastric emptying of solids, consistent with previous

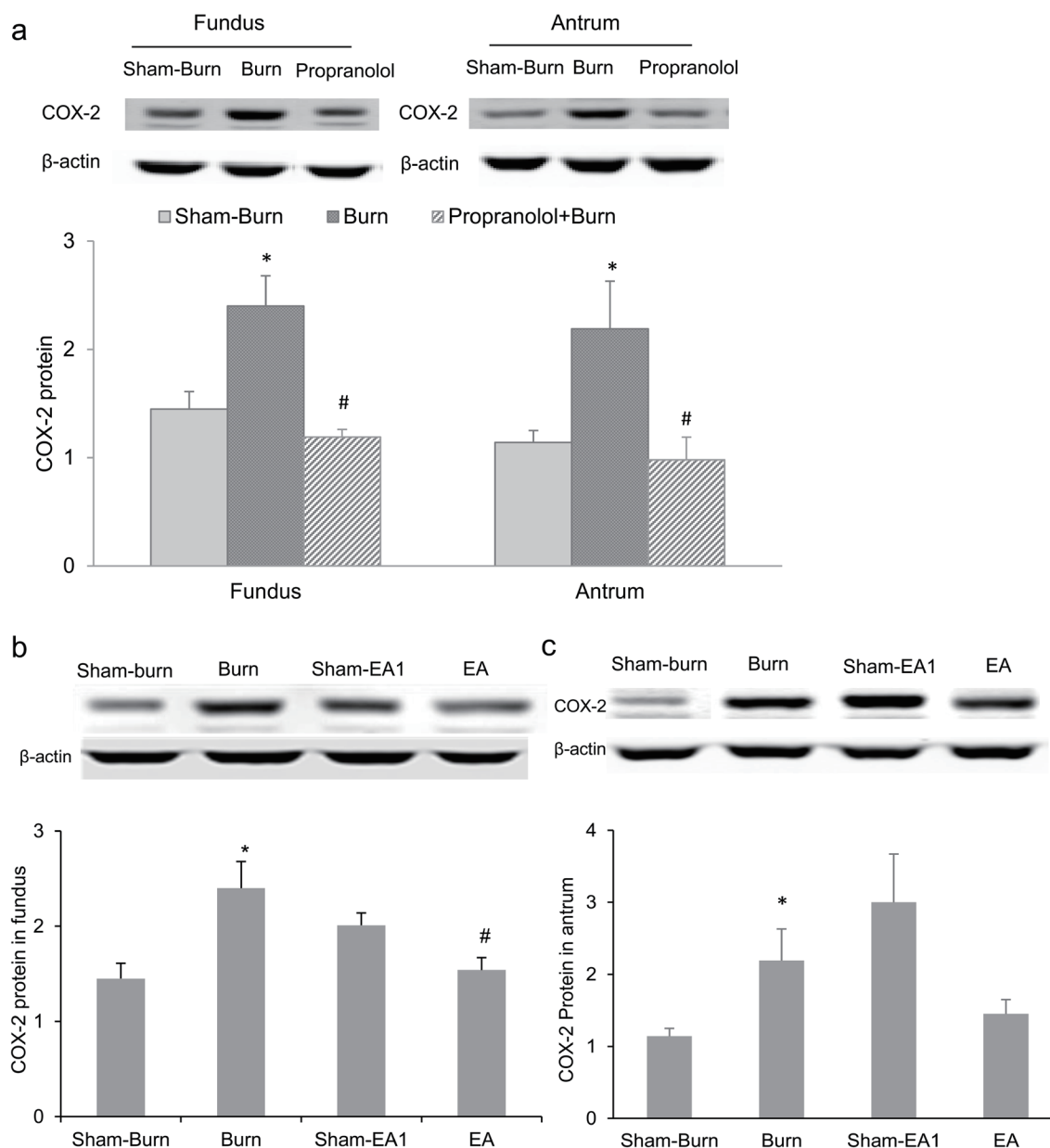


Fig. 2. Protein expressions of COX-2 in the gastric fundus and antrum in burned rats. (a) Western blots showed that COX-2 protein expression in the fundus and antrum significantly increased with burn injury (* $P = 0.01$ and 0.04 , respectively, vs. Sham-burn) and normalized with propranolol (# $P < 0.01$ and 0.04 , respectively, vs. burn). (b) EA treatment (* $P = 0.01$ vs. burn), but not Sham-EA1, decreased COX-2 protein expression in the fundus. (c) No alteration in COX-2 protein expression in the antrum was observed with EA treatment in burned rats. COX, cyclooxygenase; EA, electroacupuncture.

findings for semi-solids.^{1,2} We observed involvement of the COX-2 pathway in burn-induced delayed gastric emptying. Enhanced expression of COX-2 in the gastric fundus and antrum of burned rats was downregulated in response to selective COX-2 blockade. Our group previously implicated COX-2 in the burn-induced delay of gastric emptying of semi-solids and in pathological alterations in gastric slow waves following burn injury.^{1,2} Interestingly, we found a negative correlation between gastric emptying and COX-2 mRNA in the gastric fundus, but not in the antrum. This discrepancy may be related to regional differences in the biosynthesis and pathological effects of prostaglandins within the stomach.^{8,38,39}

Numerous studies have shown varied local regulatory effects of prostaglandins on gastric slow waves and contractions when applied regionally as exogenous prostaglandins or antagonists.^{38,40} However, whether such effects extend to more complex processes such as gastric emptying remains to be explored.

The systemic inflammatory response following burn injury is well-documented.^{41–43} It is characterized by alterations in various cytokine levels. In this study, we observed markedly elevated plasma IL-6 and IL-10 in burned rats. IL-6, a key pro-inflammatory cytokine, plays a crucial role in the development of burn-induced inflammatory cascades and is used as a predictor of severity and

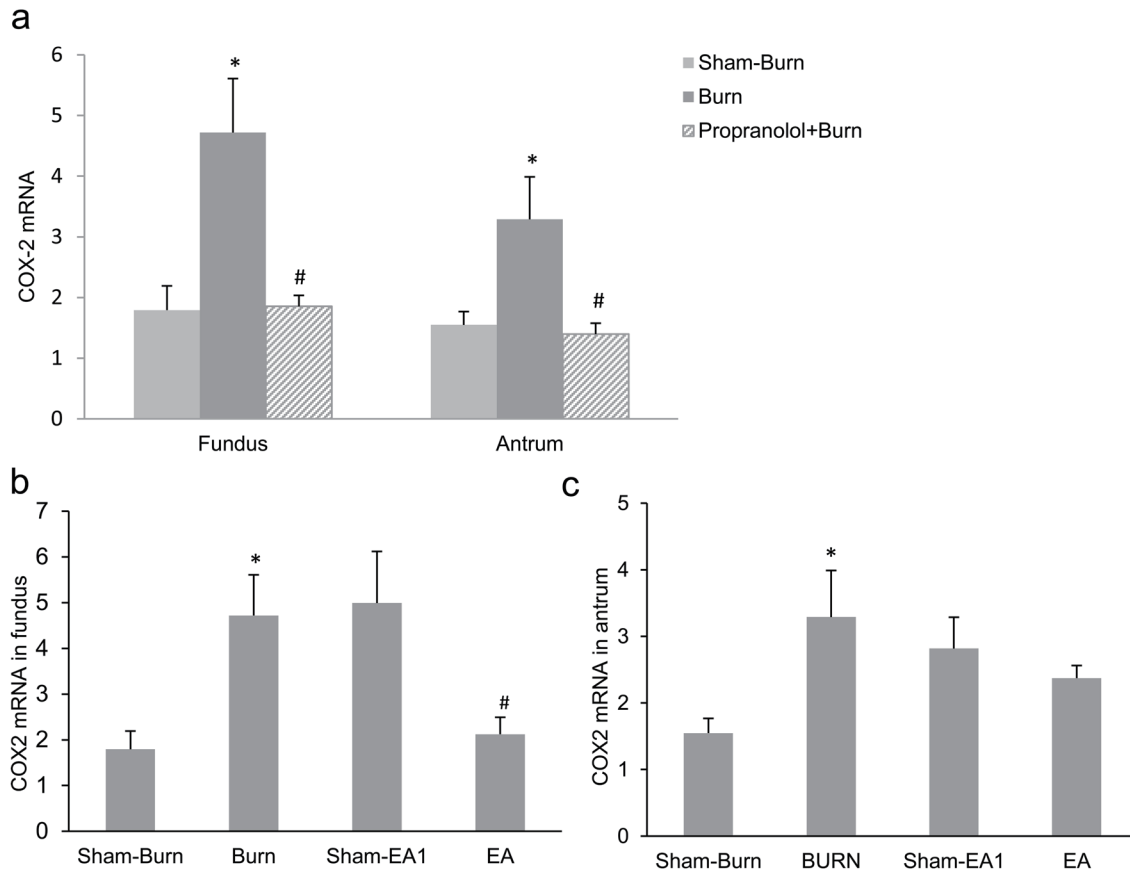


Fig. 3. COX-2 mRNA in the gastric fundus and antrum in burned rats. (a) The relative amount of COX-2 mRNA in the fundus and antrum significantly increased with burn injury (* $P = 0.01$ and 0.04 , respectively, vs. Sham-burn) and normalized with propranolol (# $P = 0.01$ and 0.03 , respectively, vs. burn). (b) EA treatment, but not Sham-EA1, reduced the burn-induced increase in COX-2 mRNA in the fundus (# $P = 0.02$ vs. burn). (c) No alteration in COX-2 mRNA in the antrum was found after EA treatment. COX, cyclooxygenase; EA, electroacupuncture.

mortality in patients with severe burn injuries.⁴³ Excessive IL-6 elevation also induces the release of the anti-inflammatory cytokine IL-10,⁴⁴ which plays a pivotal role in immunosuppression after burn injury.⁴⁵ The role of cytokine alterations in gastrointestinal dysmotility has been described in the literature. Exogenous recombinant human IL-1 α or IL-6 has been shown to reduce food intake and gastric emptying.^{46,47} Intestinal expression of IL-1 β has been implicated in burn-induced delayed intestinal transit.⁴⁸ Our study similarly showed that elevated plasma IL-6 significantly correlated with impaired gastric emptying. However, we did not directly examine the effects of either IL-6 or IL-10 on gastric emptying, nor did we assess cytokine expression in gastric tissues. Further studies are needed to define the contributions of the post-burn systemic inflammatory response to gastric emptying.

Following a severe burn injury, the sympathetic nervous system was found to be markedly activated, as demonstrated by an increase in plasma NE levels and the LF component in the spectrum of the HRV signal. The interaction between sympathetic hyperactivity and COX-2 or prostaglandins has been reported in recent years. Studies have shown that NE induces the production of prostaglandins in the superior cervical ganglia and gastric epithelial cells.^{15,49} Moreover, COX-2 and prostaglandins contribute to the activation of the sympathetic system as well.^{50,51} In the current study, to explore the relationship between sympathetic activity, COX-2 expression, and gastric emptying, we administered propranolol or phentolamine in

burned rats. Only propranolol improved the delayed gastric emptying and concurrently normalized elevated COX-2 expression in gastric tissues. Therefore, it is likely that burn-induced delayed gastric emptying involves the COX-2 mechanism mediated via the beta- but not the alpha-adrenergic pathway. We further confirmed the role of COX-2 in sympathetic system activation in burned rats, as demonstrated by the decrease in plasma NE with the administration of a selective COX-2 inhibitor.

There are known interactions between the sympathetic system and the immune system. Accordingly, we investigated the involvement of the sympathetic system in the burn-induced inflammatory response in this animal model. As expected, the post-burn elevated plasma IL-6 was reduced by propranolol treatment. Similar results have been reported in other animal stress models where propranolol blocked epinephrine-induced high plasma IL-6.¹⁷ In severely burned children, propranolol combined with human growth hormone treatment was shown to decrease serum IL-6 and IL-8 levels.¹⁸ These findings emphasize the interactions between the sympathetic system and the immune system, suggesting that the release of IL-6 in response to stress (e.g., burn) is mediated via the β -adrenergic pathway.

One of the major findings of this study is that EA at ST36 improved the burn-induced delay in gastric emptying of solids. The effects of EA at ST36 on gastric motility have been previously reported in both animals and humans. In a rodent model of func-

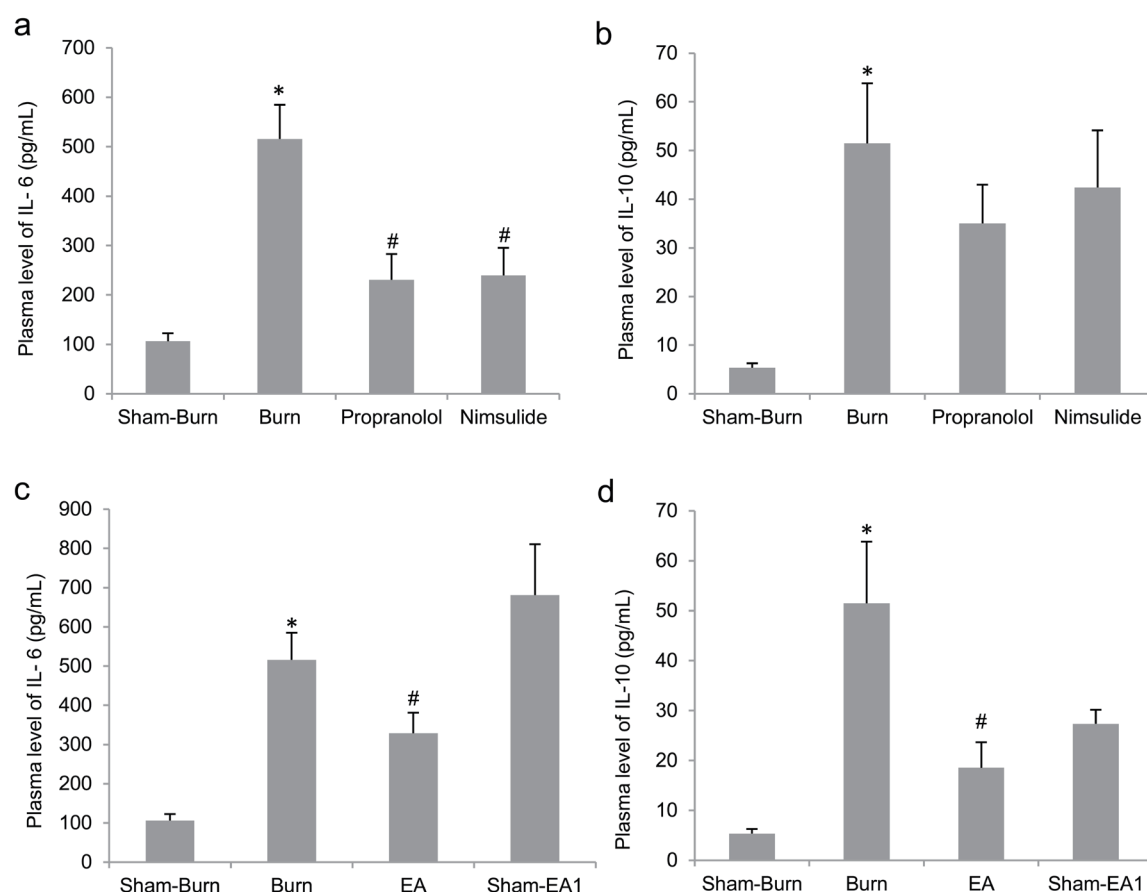


Fig. 4. Plasma levels of cytokines in burned rats. (a) Plasma IL-6 significantly increased in burned rats ($*P < 0.01$ vs. Sham-burn) and decreased after administration of propranolol or nimsulide ($^{\#}P < 0.01$ vs. burn). (b) Plasma IL-10 significantly increased in burned rats ($*P < 0.01$ vs. Sham-burn) and did not change after administration of propranolol or nimsulide. (c) EA significantly reduced the burn-induced increase in plasma IL-6 ($^{\#}P < 0.05$ vs. burn). (d) EA significantly reduced the burn-induced increase in plasma IL-10 ($^{\#}P = 0.03$ vs. burn). EA, electroacupuncture; IL, interleukin.

tional dyspepsia, EA at ST36 normalized restraint stress-induced impairment of gastric slow waves.⁵² In patients with functional dyspepsia, EA improved gastric accommodation and accelerated the gastric emptying of solids.^{25,53}

Although EA is known to improve gastric motility under various conditions, little is known about its mechanism of prokinetic action. Previous studies in our lab and others have indicated that the prokinetic effects of EA on gastric motility can be attributed to the enhancement of vagal activity in both animals and humans.^{29,30,52,54-56} In our present study, similar findings were observed in burned rats. EA at ST36 enhanced vagal activity, reflected by an elevation in the HF component of HRV and an increase in plasma PP, leading to an improvement in the burn-induced sympathovagal imbalance.

Another important and innovative aspect of this study is the investigation of the molecular mechanisms of EA on burn-induced delay in gastric emptying. EA treatment was found to reduce the burn-induced increase in COX-2 expression in the gastric fundus, and COX-2 mRNA levels were significantly correlated with gastric emptying. These findings demonstrate for the first time that the prokinetic effect of EA on gastric emptying is associated with down-regulation of COX-2. Our results align with reported inhibitory effects of EA on COX-2 expression in other animal models;^{57,58} for instance, EA at ST36 and SP6 reduced elevated COX-2

in the spinal cord after spinal nerve ligation in rats and decreased COX-2 and prostaglandin E2 levels in both paws and spinal cord in carrageenan-injected rats.

The inhibitory effect of EA on pro-inflammatory cytokines has been consistently reported.^{59,60} This effect may be attributed to EA's stimulatory effects on vagal activity, as a vagal anti-inflammatory pathway is well-documented.^{61,62} In a rodent burn model, peripheral vagal nerve stimulation was shown to reduce burn-induced elevated pro-inflammatory cytokines in serum and organ homogenates.⁶³ Similarly, our results demonstrated a marked reduction in burn-induced elevation of plasma IL-6 in response to EA treatment. These findings support the involvement of an autonomic-immunological pathway in the effects of EA on gastric emptying.

There are some limitations to the current study. According to the 2022 Burn Injury Summary Report from the American Burn Association, the prevalence of burn injury in females is 34%. We excluded female rats to avoid the hormonal effects on gastrointestinal motility; however, female rats should be included in further studies for clinical relevance. Additionally, a set of EA parameters known to enhance GI motility was selected in this study. It has been reported that EA at 5 Hz suppresses pro-inflammatory cytokines in a rodent model of colitis.⁶⁴ Parameter optimization will be needed in further EA studies for GI dysmotility induced by burn injury.

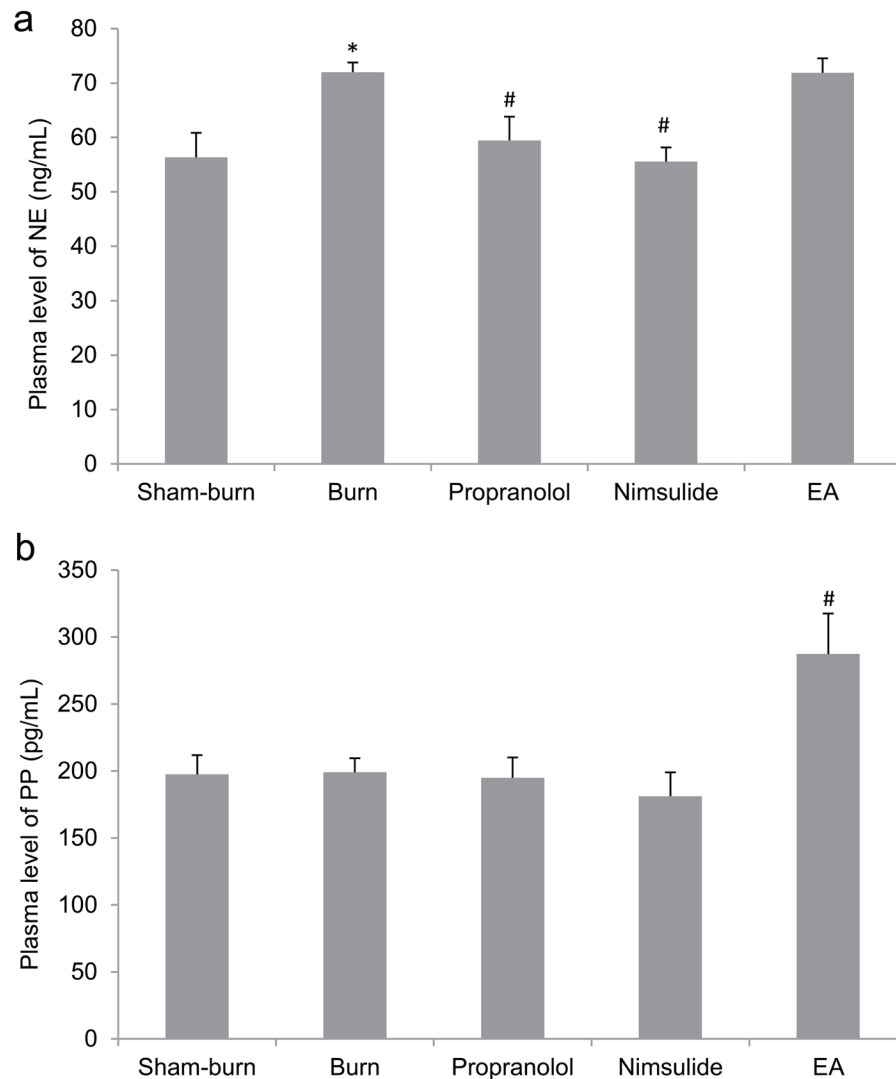


Fig. 5. Plasma levels of NE and PP in burned rats. (a) Plasma NE increased 6 h after burn injury (* $P = 0.01$ vs. sham burn) and was reduced with administration of propranolol or nimsulide (# $P = 0.01$ and <0.001 , respectively, vs. burn). No alteration in plasma NE was found after EA treatment. (b) There was no difference in plasma PP between the burn and sham burn groups. EA significantly increased plasma PP in burned rats (# $P = 0.02$ vs. burn). EA, Electroacupuncture; NE, norepinephrine; PP, polypeptide.

Conclusions

The present study suggests that burn injury impairs gastric motility by up-regulating pro-inflammatory cytokines and COX-2 via the sympathetic pathway. EA at ST36 improves burn-induced delay in gastric emptying by down-regulating COX-2 and pro-inflammatory cytokines through vagal enhancement.

Acknowledgments

The study was done at University of Teax and Zhaohui Zhang is now with Department of Acupuncture, Nanjing Medical University Affiliated Hospital, Nanjing, China.

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Conflict of interest

Jiande Chen has served as Executive Editor-in-Chief of the *Journal of Translational Gastroenterology* since January 2023. Jieyun Yin is an employee of Transtimulation Research Inc. The other authors declare no conflicts of interest.

Author contributions

Study concept and design (HL, JC, JY), acquisition of data (HL, ZZ, JY), analysis and interpretation of data (HL), drafting of the manuscript (HL), critical revision of the manuscript for important

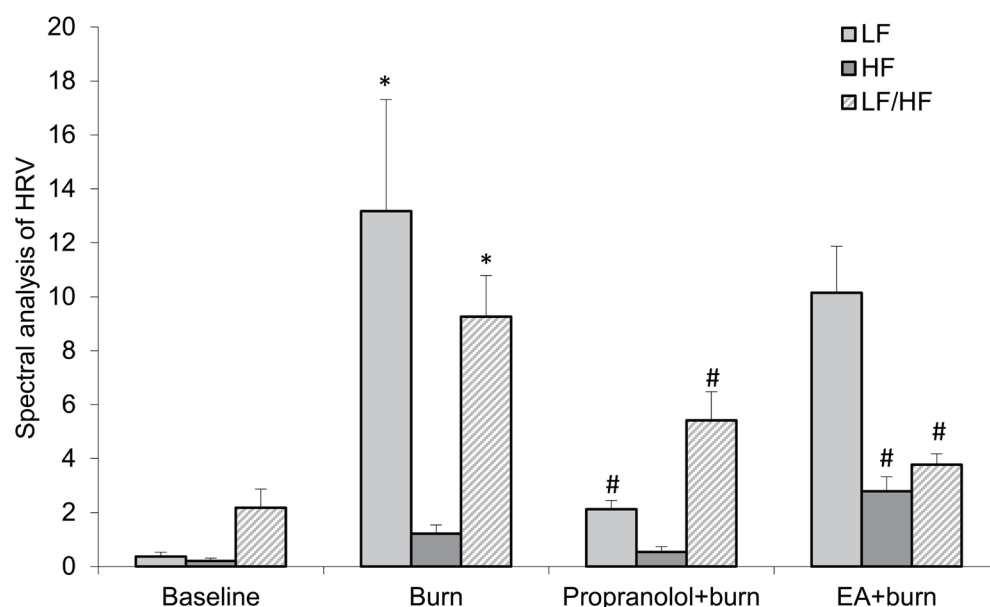


Fig. 6. Autonomic function in burned rats. The results of HRV analysis showed that the LF (sympathetic activity) and the ratio of LF/HF (sympathovagal balance) significantly increased with severe burn injury compared to sham burn (* $P < 0.01$) and decreased with administration of propranolol (# $P < 0.05$ vs. burn). EA enhanced vagal activity (HF) and improved sympathovagal balance (LF/HF) in burned rats (# $P < 0.05$ vs. burn). EA, electroacupuncture; HRV, Heart rate variability.

intellectual content (HL, ZZ, JC), statistical analysis (HL), technical support (HSS, JY), and preparation of manuscript (HL, JY, JC).

Ethical statement

The animal study was performed in compliance with the guidelines of the National Institutes of Health and approved by the Animal Care and Use Committee of the University of Texas Medical Branch. All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering.

Data sharing statement

Data in excel format and information on statistical analysis used in support of the findings of this study are available from the corresponding author at jiandedzchen@gmail.com upon request.

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